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GWAS-identified colorectal cancer susceptibility locus associates with disease prognosis

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ABSTRACT

Purpose: Extensive evidence has suggested that risk factors of cancer development may also modulate cancer clinical outcome. Recent genome-wide association (GWA) studies identified several single nucleotide polymorphisms (SNPs) predisposing to colorectal cancer (CRC). Given the pivotal importance of these variants in CRC, we sought to evaluate their associations with clinical outcomes of the disease.

Experimental Design: In a well-characterised cohort including 380 Chinese CRC patients, we genotyped seven SNPs identified in previous multi-stage GWA studies and analysed their associations with patient recurrence and survival.

Results: One SNP on chromosome 15q13, rs4779584 was associated with reduced risk of death with a hazard ratio (HR) of 0.33 (95% confidence interval [CI] 0.15–0.72, $P = 0.007$). Another SNP in a gene-desert region on chromosome 10p14, rs10795668, was associated with a reduced risk of recurrence with an HR of 0.55 (95% CI 0.30–1.00, $P = 0.05$). In a stratified analysis, this association was only evident in patients receiving chemotherapy (HR = 0.32, 95% CI 0.14–0.78, $P = 0.01$, log rank $P = 0.004$), but not in those without chemotherapy (HR = 1.08, 95% CI 0.43–2.73, $P = 0.87$, log rank $P = 0.66$). Moreover, we found that the effects of chemotherapy on CRC recurrence was only evident in patients with the variant-containing genotypes (HR = 0.35, 95% CI 0.13–0.94, $P = 0.04$) but not in those with the wild-type genotype of rs10795668. Further analyses indicated a borderline significant interaction effect (P interaction = 0.05) between rs10795668 and chemotherapy on patient recurrence.

Conclusions: Our data suggested that rs10795668, a CRC susceptibility variant identified by GWA studies, might be used as a biomarker to identify CRC patients with high risk of recurrence after chemotherapy.

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1. Introduction

Colorectal cancer (CRC) is a common malignancy as well as a leading cause of cancer mortality in both Europe and China.[1–3] In both regions, the incidence rate of CRC has been increasing since 1990s, especially in males.[2,3] In 2008, CRC had the highest incidence among all cancers in Europe, with approximately 436,000 new cases diagnosed.[1] In addition, it also caused the death of 212,000 patients, which was only less than the number of deaths from lung cancer.[1] Nonetheless, the mortality rate of CRC has decreased in Europe whereas kept flat in China in recent years, possibly due to the early screening and detection, as well as the use of more advanced surgical and systemic modalities.[2,4] However, despite the improvement in mortality, the 5-year survival rate of CRC remained very high in several northern European countries and many urban areas of China.[2,4] Moreover, a considerable portion of successfully treated CRC patients develop a recurrence or metastasis within 5 years of diagnosis, highlighting the importance of the development of novel biomarkers to identify those patients who are most likely to recur and progress to receive more aggressive therapies.[5,6]

Emerging evidence has shown that single nucleotide polymorphisms (SNPs) may be used as surrogates of patients' genetic backgrounds to predict therapeutic response and prognosis of cancer.[7] Previous studies have identified numerous genetic variations as predictors of CRC risk.[8] However, whether these variations have any influence on CRC clinical outcomes remains unclear. It has been suggested that genomic variations and aberrations affecting cancer development may also influence the clinical outcome of the same diseases.[9] This notion is supported by numerous epidemiological studies of various cancers. For instance, the *p53* codon 72 and *MDM2* SNP309 have been associated with both the aetiology and prognosis of aerodigestive tract cancers in various independent studies.[10,11] Multiple SNPs in DNA damage response and repair pathway genes also affect the risk and clinical outcome of a wide array of tobacco-related cancers.[12,13] In CRC, the A870G SNP, a well-studied variation in *CCND1* gene that modulates the alternative splicing of CCND1 messenger RNA, has been associated with both the risk and the prognosis of CRC patients in various independent studies.[14–16] These observations may be partially explained by the fact that the majority of oncogenic pathways implicated in the initiation of malignancies are also important for the progression as well as the clinical characteristics of tumours after diagnosis. Moreover, not only genetic variations, but also a wide spectrum of biomarkers based on epigenetic changes, structural variations, copy number variations, loss of heterozygosity and somatic mutations, etc., can be used as predictors of both cancer risk and clinical outcomes.[9] In addition, a plethora of independent studies have reached the same conclusion in CRC patients, as previously reviewed.[17,18]

Recent multi-stage genome-wide association (GWA) studies have identified several highly robust SNPs that are significantly associated with CRC risk.[19–22] However, the effects of these variations on the clinical outcomes of CRC patients have not been evaluated. In this study, we sought to assess the effects of eight highly robust susceptibility loci identified in CRC GWA studies with CRC recurrence and death in a cohort of 380 Chinese CRC patients. We also evaluated the effects of the significant variants on chemotherapy, one of the mainstream CRC therapies.

2. Materials and methods

2.1. Study population

This study included a population of incident CRC patients enrolled in the Xijing Hospital and Tangdu Hospital affiliated with the Fourth Military Medical University (FMMU) in Xi'an, China. All patients were Han Chinese with newly diagnosed and histologically confirmed CRC. There were no restrictions on age, gender, cancer stage on enrolment, except for history of other cancers. The patient enrolment started in August 2008 with the retrospective enrolment of patients diagnosed as early as February 2006. As of January 2010, 465 CRC patients have been enrolled with complete demographic and clinical information. Among them, 380 patients have complete and validated follow-up data on recurrence and death. Informed consent was obtained for each patient. The research was approved by local research ethics committees of participating institutes.

2.2. Epidemiologic and clinical data

For each patient, demographic data were collected through in-person interview at the time of initial visit or follow-up in the clinics, medical chart review or consultation with the treating physicians. Data acquired from multiple sources were compared and validated. If discrepancies were identified, patients were further contacted for verification. An individual who smoked more than 100 cigarettes was defined as an ever smoker, otherwise as a never smoker. Detailed clinical information was collected through medical chart review or consultation with treating physicians. At 6-month intervals, a trained clinical specialist updated follow-up information on recurrence and death through on-site interview, direct calling, or medical chart review. The latest follow-up data in this analysis were obtained in February 2010. Among the currently enrolled patients, the percentage of patient loss during follow-up was less than 10%. For patient enrolled after August 2008, 5-ml of blood was available for genomic DNA extraction in the laboratory. For patients enrolled before August 2008, genomic DNA was extracted from approximately 100 mg of adjacent normal tissues obtained by a pathologist after surgery. Tumour and adjacent normal tissues were available for 71% of all the patients in this study.

2.3. Gene/polymorphism selection and genotyping

The candidate polymorphisms were selected based on review of recent CRC GWA studies.[19–22] All SNPs have been identified in multi-stage GWA studies as *bona fide* CRC susceptibility loci. In this study, a total of eight SNPs were genotyped in the 380 patients with complete and validated follow-up recurrence and survival data, using the Sequenom iPLEX genotyping

system (Sequenom Inc., CA). The eight SNPs include rs3802842, rs4779584, rs6983267, rs7014346, rs10795668, rs16892766, rs4939827 and rs12953717. Laboratory personnel conducting genotyping were blinded to patient information. Strict quality control measures were implemented during genotyping with over 99% concordance with the main genotyping results.

2.4. Statistical and bioinformatics analysis

The primary endpoint for this study was the time to recurrence, which was defined as the time from initial treatment to local recurrence and/or distant metastasis. All patients without recurrence were censored for the analysis. Recurrence was confirmed through the combined evaluations of imaging findings (ultrasound, computed tomography, positron emission tomography, magnetic resonance imaging) and laboratory results (mainly the carcinoembryonic antigen test). The secondary endpoint was overall patient survival, which was defined as the time between initial treatment to death from any cause. In addition, event-free survival time was also assessed, which was defined as the time from initial treatment to local recurrence, distant metastasis, death from any cause or to the date of last follow-up. Kaplan–Meier curve and log-rank test were used to assess the differences in time to recurrence, death or any event (recurrence and/or death). Hazard ratios (HRs) were estimated from a multivariate Cox proportional hazards model, adjusting for age, gender, smoking status, tumour position, tumour differentiation, tumour stag, and chemotherapy, where appropriate. SAS (version 9.1, SAS Institute Inc, Cary, NC) and STATA software packages (version 8, STATA Corp., College Station, TX) were used for the above mentioned statistical analyses. All P values in this study were two-sided. $P \leqslant 0.05$ was considered the threshold of statistical significance. Bioinformatics analyses of the region harbouring rs10795668 were done using the UCSC Genome Browser (http://genome.ucsc.edu)[23] to search functional elements and using the MultiZ and the PhyloP programmes[24] to compare the degree of conservation of the region across 46 vertebrates.

3. Results

3.1. Characteristics of the study population

Table 1 summarises the distribution of demographic and pathoclinical characteristics of the 380 CRC patients included in this study. The mean age at the time of diagnosis was 59.3 (range, 22–90) years. There were 212 (55.8%) male patients. The majority of patients were never smokers (75.8%) and had adenocarcinoma (95.5%). There were approximately equal number of patients with colon cancer (49.5%) and rectal cancer (50.5%). About 46.6% of patients had stage 2 disease, while stage 0, 1, 3 and 4 diseases were present in 2.9%, 12.4%, 26.1% and 11.3% of the patients, respectively. The majority (65.3%) of patients had moderately differentiated tumours. Almost all (97.9%) patients underwent surgery after diagnosis. No patient received radiotherapy or targeted therapies, but half (50.5%) of the patients received adjuvant chemotherapy after surgery. Among the 192 patients receiving chemotherapy, 185 (96.4%) received the FOLFOX regimen including folinic acid (FOL), 5′ fluorouracil (F) and oxaliplatin (OX). The median follow-up time for the 380 patients included in this study was 14.2 months. During the follow-up period, there were 51 (13.4%) patients who developed recurrences and 47 (12.4%) patients who have died. There were 83 (21.8%) patients who had at least one event (recurrence and/or death).

Table 1 – Demographic and clinicopathological characteristics of the 380 patients with colorectal cancer.

Variables	Number of patients (%) N = 380
Age, average (range) (in years)	59.3 (22–90)
Gender	
Male	212 (55.8%)
Female	168 (44.2%)
Smoking status	
Ever	92 (24.2%)
Never	288 (75.8%)
Tumour position	
Colon	188 (49.5%)
Rectum	192 (50.5%)
Tumour histology	
Adenocarcinoma	363 (95.5%)
Other	17 (4.5%)
Tumour stage	
0	11 (2.9%)
1	47 (12.4%)
2	177 (46.6%)
3	99 (26.1%)
4	43 (11.3%)
Unknown	3 (0.7%)
Tumour differentiation	
Poor	32 (8.4%)
Moderate	248 (65.3%)
Well	83 (21.8%)
Unknown	17 (4.5%)
Surgery	
Yes	372 (97.9%)
No	8 (2.1%)
Chemotherapy	
Yes	192 (50.5%)
No	187 (49.2%)
Unknown	1 (0.3%)
Recurrence	
Yes	51 (13.4%)
No	329 (86.6%)
Death	
Yes	47 (12.4%)
No	329 (86.6%)
Unknown	4 (1.0%)
Event (recurrence and/or death)	
Yes	83 (21.8%)
No	297 (78.2%)

3.2. Main effects and stratified analyses by individual polymorphisms

The majority of our patients had adenocarcinoma (95.5%) and received surgery resection (97.9%). In addition, 96.4% of

Table 2 – Association of GWAS SNPs with clinical outcome of colorectal cancer patients.

SNP	Chromosome region	Genotype[a]	Recurrence/total	HR (95% CI)[b]	P value	Death/total	HR (95% CI)[b]	P value	Event/no event[c]	HR (95% CI)[b]	P value
rs3802842	11q23	WW	12/103	1		9/100	1		71/103	1	
		WV + VV	33/247	1.08 (0.55–2.13)	0.81	31/246	1.24 (0.57–2.71)	0.58	56/247	1.20 (0.69–2.10)	0.52
rs4779584	15q13	WW	32/227	1		32/225	**1**		54/227	**1**	
		WV + VV	14/122	0.67 (0.35–1.28)	0.23	9/120	**0.33 (0.15–0.72)**	**0.005**	20/122	**0.49 (0.29–0.92)**	**0.008**
rs6983267	8q24	WW	12/110	1		11/110	1		19/110	1	
		WV + VV	34/241	1.19 (0.61–2.33)	0.60	30/237	0.99 (0.48–2.02)	0.97	55/243	1.14 (0.67–1.94)	0.63
rs7014346	8q24	WW	20/179	1		21/176	1		35/179	1	
		WV + VV	25/171	1.24 (0.68–2.27)	0.49	19/170	0.69 (0.36–1.36)	0.29	38/171	1.00 (0.62–1.61)	0.99
rs10795668	10p14	WW	25/149	**1**		21/146	1		38/149	1	
		WV + VV	20/198	**0.55 (0.30–1.00)**	**0.05**	19/197	0.74 (0.38–1.42)	0.36	35/198	0.75 (0.46–1.22)	0.25
rs4939827	18q21	WW	29/207	1		26/205	1		46/207	1	
		WV + VV	17/143	0.86 (0.47–1.59)	0.64	15/141	0.86 (0.45–1.64)	0.64	28/143	0.89 (0.55–1.44)	0.65
rs12953717	18q21	WW	31/215	1		27/213	1		48/215	1	
		WV + VV	14/134	0.72 (0.38–1.37)	0.32	13/132	0.78 (0.40–1.53)	0.47	25/134	0.84 (0.51–1.38)	0.50

Note: The significant P value (less than 0.05) was in bold.

[a] WW, homozygous wild-type genotype; WV, heterozygous genotype; VV, homozygous variant genotype.

[b] Adjusted for age, gender, smoking status, tumour position, tumour differentiation, tumour stage, and chemotherapy.

[c] *Event:* recurrence and/or death.

chemotherapy-treated patients received the FOLFOX regimen. Therefore, in order to eliminate the confounding effects of these variables and to further enhance the homogeneity of our population, we excluded those patients who did not have adenocarcinoma, did not receive surgery or received non-FOLFOX chemotherapy agents. We had 351 patients left for downstream analyses. We compared the host characteristics of the patients with and without chemotherapy (Supplementary Table 1). Except for age ($P = 0.01$), no other host characteristic exhibited significant difference between patients with and without chemotherapy. For the eight SNPs genotyped in this study, the average genotyping call rate is 99.5% (98.7–100%). One SNP, rs16892766, was excluded from further analyses due to small minor allele frequency (MAF) (<2%). The associations of the remaining seven SNPs with CRC recurrence, death and event are listed in Table 2. Only a dominant genetic model (variant-containing genotypes versus homozygous wild-type genotype) was tested to obtain the optimum statistical power. Among the seven SNPs, rs10795668, a SNP located on chromosome 10p14 exhibited a statistically significant association with CRC recurrence. Compared to the homozygous wild-type (WW) genotype, the variant-containing (WV + VV) genotypes were associated with a significantly reduced recurrence risk, with an HR of 0.55 (95% CI 0.30–1.00, $P = 0.05$) (Table 2). rs4779584, a SNP located on chromosome 15q13, was associated with a significantly reduced risk of death, with an HR of 0.33 (95% CI 0.15–0.72, $P = 0.005$). In addition, this SNP was inversely associated with the risk of occurrence of event (recurrence and/or death) with an HR of 0.49 (95% CI 0.29–0.92, $P = 0.008$) (Table 2). Except for two patients for whom we did not have information on the reason of their deaths, all other patients in this study had CRC-specific deaths. Analyses excluding those two patients yielded almost the same results (data not shown).

3.3. *Stratified analyses of rs10795668 and rs4779584*

In stratified analyses, the recurrence risk conferred by rs10795668 remained significant in patients with moderate tumour differentiation (HR = 0.36, 95% CI 0.17–0.77, $P = 0.008$), patients whose DNA was from blood (HR = 0.45, 95% CI 0.22–0.92, $P = 0.03$), and patients receiving chemotherapy (HR = 0.32, 95% CI 0.14–0.78, $P = 0.01$) (Table 3). The death risk conferred by rs4779584 remained significant in old patients (HR = 0.36, 95% CI 0.13–1.00, $P = 0.05$), females (HR = 0.11, 95% CI 0.02–0.53, $P = 0.006$), never smokers (HR = 0.18, 95% CI 0.06–0.53, $P = 0.002$), patients with rectal cancer (HR = 0.22, 95% CI 0.06–0.81, $P = 0.02$), patients with moderate tumour differentiation (HR = 0.27, 95% CI 0.09–0.83, $P = 0.02$), non-stage 4 patients (stage 0, 1, 2, and 3) CRC (HR = 0.30, 95% CI 0.11–0.81, $P = 0.02$) and patients whose DNA was from blood (HR = 0.25, 95% CI 0.08–0.82, $P = 0.02$) (Table 3).

3.4. *rs10795668 Interacts with chemotherapy and modulates the effects of chemotherapy on CRC recurrence*

Because the rs10795668-conferred reduction in CRC recurrence remained significant in patients receiving chemotherapy but not in those without chemotherapy, we further evaluated the modulating effects of chemotherapy on CRC

Table 3 – Association of rs10795668 and rs4779584 with clinical outcome of colorectal cancer patients stratified by host characteristics.

Variables	Stratum	Genotype[a]	Recurrence/ total	rs10795668 HR (95% CI)[b]	P value	Death/ total	rs4779584 HR (95% CI)[b]	P value
Overall		WW	25/149	**1**		32/225	**1**	
		WV + VV	20/198	**0.55 (0.30–1.00)**	**0.05**	9/120	**0.33 (0.15–0.72)**	**0.005**
Age	Young (⩽61)	WW	11/73	1		17/117	1	
		WV + VV	10/94	0.56 (0.22–1.47)	0.68	3/49	0.41 (0.11–1.52)	0.18
	Old (>61)	WW	14/76	1		15/108	1	
		WV + VV	10/104	0.42 (0.17–1.03)	0.06	6/71	**0.36 (0.13–1.00)**	**0.05**
Gender	Male	WW	15/90	1		14/124	1	
		WV + VV	13/101	0.78 (0.35–1.76)	0.56	7/65	0.45 (0.16–1.28)	0.13
	Female	WW	10/59	1		18/101	**1**	
		WV + VV	7/97	0.36 (0.13–1.06)	0.06	2/55	**0.11 (0.02–0.53)**	**0.006**
Smoking status	Never smoker	WW	2/114	1		27/172	**1**	
		WV + VV	17/147	0.56 (0.28–1.12)	0.10	4/89	**0.18 (0.06–0.53)**	**0.002**
	Ever smoker	WW	5/35	1		5/53	1	
		WV + VV	3/51	0.54 (0.10–2.81)	0.47	5/31	0.50 (0.09–2.65)	0.41
Tumour position	Colon	WW	10/67	1		17/108	1	
		WV + VV	9/106	0.60 (0.23–1.57)	0.30	5/61	0.37 (0.13–1.09)	0.07
	Rectum	WW	15/82	1		15/117	**1**	
		WV + VV	11/93	0.51 (0.22–1.15)	0.11	8/59	**0.22 (0.06–0.81)**	**0.02**
Tumour differentiation	Moderate	WW	18/84	**1**		19/157	**1**	
		WV + VV	12/148	**0.36 (0.17–0.77)**	**0.008**	4/77	**0.27 (0.09–0.83)**	**0.02**
	Poor	WW	2/17	**1**		8/19	**1**	
		WV + VV	2/12	NA[c]	NA[c]	0/9	NA[c]	NA[c]
	Well	WW	5/43	1		4/46	1	
		WV + VV	6/37	1.38 (0.38–5.08)	0.63	5/33	2.28 (0.41–12.64)	0.35
Tumour stage	Stage 0–3	WW	19/132	1		22/197	**1**	
		WV + VV	20/176	0.75 (0.39–1.46)	0.40	5/109	**0.30 (0.11–0.81)**	**0.02**
	Stage 4	WW	6/17	1		10/28	1	
		WV + VV	0/22	NA[c]	NA[c]	4/11	0.43 (0.09–1.97)	0.28
Source of DNA	Blood	WW	21/124	**1**		20/187	**1**	
		WV + VV	12/161	**0.45 (0.22–0.92)**	**0.03**	4/96	**0.25 (0.08–0.82)**	**0.02**
	Tissue	WW	4/25	1		12/38	1	
		WV + VV	8/37	1.33 (0.27–6.65)	0.73	5/24	0.35 (0.10–1.18)	0.09
Chemotherapy	Yes	WW	16/73	**1**		16/114	1	
		WV + VV	8/108	**0.32 (0.14–0.78)**	**0.01**	4/64	0.31 (0.09–1.01)	0.06
	No	WW	9/75	1		16/111	1	
		WV + VV	12/90	1.08 (0.43–2.73)	0.87	5/55	0.35 (0.11–1.08)	0.07

Note: The significant *P* value (less than 0.05) was in bold.

[a] WW, homozygous wild-type genotype; WV, heterozygous genotype; VV, homozygous variant genotype.

[b] Adjusted for age, gender, smoking status, tumour position, tumour differentiation, tumour stage, and chemotherapy, where appropriate.

[c] NA, not available.

recurrence by rs10795668. Kaplan–Meier curves indicated a borderline statistical significance in the differences between time to recurrence of patients with the wild-type and variant-containing genotypes (Log rank $P = 0.08$, Fig. 1A). However, the differences were more evident in patients receiving chemotherapy (Log rank $P = 0.004$, Fig. 1B), but not in those without chemotherapy (Log rank $P = 0.66$, Fig. 1C). We further assessed the effects of chemotherapy on CRC recurrence in all patients, as well as in patients stratified by rs10795668 (Table 4). We found that overall, chemotherapy was not significantly associated with patient recurrence (HR = 0.70, 95% CI 0.38–1.28, $P = 0.25$). However, chemotherapy exhibited a significant favourable effect on recurrence in patients with the variant-containing genotype of rs10795668 (HR = 0.35, 95% CI 0.13–0.94, $P = 0.04$), but not in patients with the wild-type genotype of rs10795668 (HR = 1.18, 95% CI 0.51–2.76, $P = 0.70$). Furthermore, significant interaction effects between rs10795668 and chemotherapy were observed in the analyses of risk for recurrence (P interaction = 0.05) and risk for event (recurrence and/or death) (P interaction = 0.04), respectively (Table 5).

3.5. *Functional prediction of rs10795668 by bioinformatics analyses*

We also conducted bioinformatics analyses of the 20 kb genomic region harbouring rs10795668 (10 kb upstream and 10 kb downstream). We used the UCSC Genome Browser to look

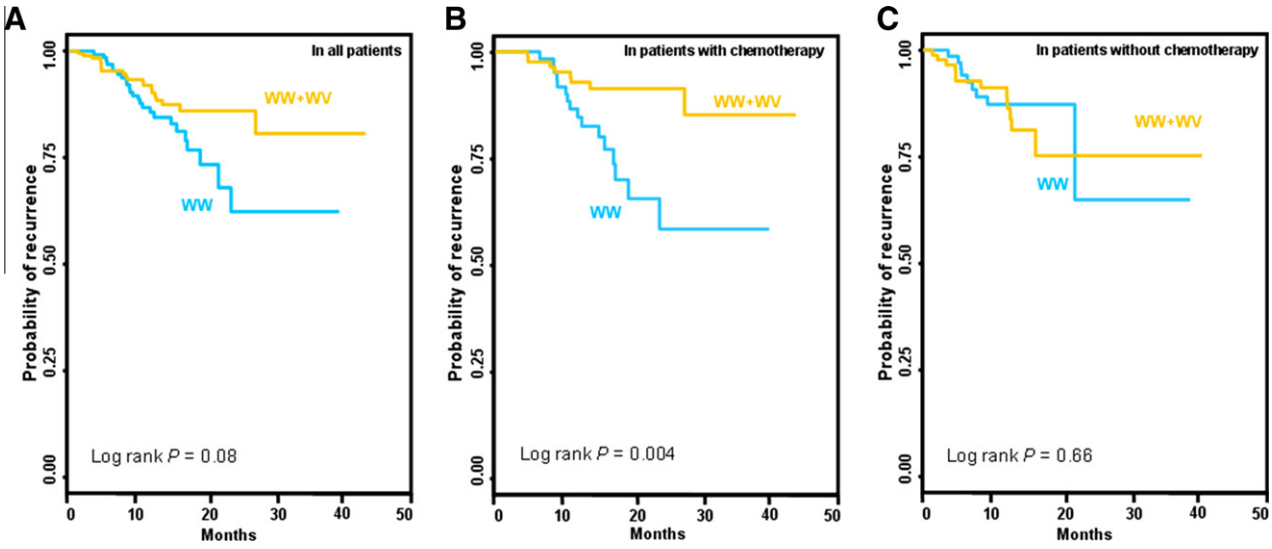

Fig. 1 – Kaplan–Meier recurrence-free curve of rs10795668 SNP in (A) all patients, (B) patients with FOLFOX chemotherapy, and (C) patients without chemotherapy.

Table 4 – Modulating effects of chemotherapy on CRC clinical outcomes by rs10795668.

	Recurrence/ total	HR (95% CI)[a]	P value
In all patients			
No chemotherapy	22/168	Reference	
Chemotherapy	24/182	0.70 (0.38–1.28)	0.25
In patients with WW[b] genotype of rs10795668			
No chemotherapy	9/75	Reference	
Chemotherapy	16/77	1.18 (0.51–2.76)	0.70
In patients with WV + VV[b] genotypes of rs10795668			
No chemotherapy	12/90	Reference	
Chemotherapy	8/108	**0.35 (0.13–0.94)**	**0.04**

Note: The significant P value (less than 0.05) was in bold.

[a] Adjusted for age, gender, smoking status, tumour position, tumour differentiation, tumour stage, and chemotherapy, where appropriate.

[b] WW, homozygous wild-type genotype; WV, heterozygous genotype; VV, homozygous variant genotype.

for annotated functional genes, miRNAs, and expressed sequence tags (ESTs), and identified a ribosomal RNA gene (AL355333.1) and several ESTs. However, none of them has been reported to be related to cancer development. We further conducted conservation analyses using MultiZ and PhyloP programmes and identified several highly conserved regions downstream of rs10795668, although no functional genes were identified within these conserved regions (data not shown).

4. Discussion

We evaluated the effects of eight genetic variations identified by recent CRC GWA studies on the clinical outcomes of a cohort of Chinese CRC patients. We found that two SNPs, rs10795668 on chromosome 10p14 and rs4779584 on 15q13, were significantly associated with CRC recurrence and survival, respectively. We also noticed significant modulating effects by rs10795668 on the effects of chemotherapy on CRC recurrence, that is, a dramatically reduced recurrence risk conferred by chemotherapy was only observed in patients with the variant-containing genotypes of rs10795668 but not in those with the wild-type genotype.

The rs10795668 SNP was firstly reported to be associated with a significantly altered risk of CRC in several European populations.[21] In a four-stage GWA study with a total of more than 35,000 subjects, rs10795668 was one of the two SNPs exhibiting significance in all four stages of the analyses. In the pooled analysis of all subjects, rs10795668 was associated with an odds ratio (OR) of 0.87 (95% CI 0.83–0.91) and 0.80 (95% CI 0.74–0.86) for heterozygous and homozygous variant genotypes, respectively[20]. The significance level was 2.5×10^{-13}, with an apparent gene-dosage effect. The variant allele of rs10795668 conferred a reduced CRC risk in the GWA study and a reduced CRC risk for recurrence in our study. This is biologically plausible because the oncogenic pathways impacted by this SNP or linked causative loci may confer a similar protective effect in both the development and treatment response of CRC. The rs10795668 SNP is located within a large linkage disequilibrium (LD) block of about 82 kb in length. Based on the original report, no known or predicted protein-coding genes are present in the 400 kb region harbouring this SNP[21], indicating that the observed association is either through long-range LD with other causative loci or other mechanisms such as chromosome instability or loss of heterozygosity in this chromosomal region.[25,26] Further bioinformatics analyses identified a ribosomal RNA gene (AL355333.1) and several ESTs in the 20 kb genomic region harbouring rs10795668, although none of them has been reported to be related to cancer development. Additional fine-mapping and functional analyses are needed to further address the physiological role of this SNP in CRC risk and clinical outcomes.

Table 5 – Joint and interaction effects between rs10795668 and chemotherapy on CRC recurrence and event-free survival.

rs10795668 genotype[a]	Chemotherapy	Recurrence/total	HR (95% CI)[b]	*P* value
WW	Yes	16/73	Reference	
WW	No	9/75	0.77 (0.34–1.78)	0.54
WV + VV	Yes	8/108	**0.32 (0.13–0.75)**	**0.009**
WV + VV	No	12/90	0.85 (0.39–1.85)	0.68
Interaction				**0.05**
rs10795668 genotype[a]	Chemotherapy	Event/total	HR (95% CI)[b]	*P* value
WW	Yes	22/73	Reference	
WW	No	16/75	0.57 (0.50–1.90)	0.93
WV + VV	Yes	15/108	**0.47 (0.24–0.91)**	**0.03**
WV + VV	No	20/90	1.23 (0.66–2.31)	0.52
Interaction				**0.04**

Note: The significant *P* value (less than 0.05) was in bold.

[a] WW, homozygous wild-type genotype; WV, heterozygous genotype; VV, homozygous variant genotype.

[b] Adjusted for age, gender, smoking status, tumour position, tumour differentiation, tumour stage, and chemotherapy, where appropriate.

In this study, we also found another SNP, rs4779584 that was associated with CRC survival. The variant-containing genotype of rs4779584 conferred a 57% reduction in risk of death. This SNP was first identified in a study searching for low-penetrance genetic loci within the region harbouring *CRAC1*, a high-penetrance gene predisposing to hereditary mixed polyposis syndrome (HMPS) which is a mendelian disease characterised by the development of colorectal polyps and CRC.[20] Through genetic fine-mapping, Broderick et al. identified a region on chromosome 15q13.3–13.4 that contains three known genes including *SCG5*, *GREM1*, and *FMN1*.[20] They further genotyped 145 SNPs in this region in 718 CRC cases with family history and/or early age onset and 960 unaffected controls. The most significant finding was rs4779584, located in the 3′ region of *SCG5* and *FMN1*, whereas in the 5′ region of *GREM1*.[19] This finding was further replicated in three other populations with a total number of 7961 CRC cases and 6803 controls (pooled analysis, significance level 4.44×10^{-14}).[20] The physiological significance of this SNP remains to be deciphered. The three surrounding genes within the region have been implicated in several oncogenic signalling pathways.[27,28] Conservation analyses also identified several highly conserved components within the 20 kb surrounding region of rs4779584 (data not shown). However, functional assays using lymphoblastoid cell lines with known genotype status of rs4779584 did not provide evidence that this SNP affects mRNA expression of these genes.[20] Further independent studies are needed to confirm our findings and functional and genetic studies are warranted to characterise the observed effects.

In the stratified analysis of rs10795668, we found that the effect of this SNP on CRC recurrence was only evident in patients receiving chemotherapy but not in those without chemotherapy (Table 3). Furthermore, in patients without chemotherapy, the variant-containing genotypes of rs10795668 seemed to confer an increased risk of recurrence, although not reaching statistical significance. When we stratified the effect of chemotherapy on CRC recurrence by rs10795668 genotypes, we found that rs10795668 significantly modified the response of patients to chemotherapy (Table 4). Although chemotherapy did not exhibit apparent therapeutic efficacy in all patients, it showed a significant effect on CRC recurrence in those patients having the variant-containing genotype of rs10795668. Similar modifying effects by genetic polymorphisms on risk stratification and therapy response have also been reported in other molecular epidemiological studies.[29,30] These findings suggested that, if validated, the genotypes of rs10795668 might be used as a potential biomarker to select those patients who are likely to have favourable response after chemotherapy.

Our study has several strengths. First, the patient population was enrolled from Xi'an and adjacent areas. This region is highly attractive in conducting population-based research because of the geographical stability with low mobility rate. Second, the patients analysed in this study were highly homogenous, in that they all had adenocarcinoma, received surgery to remove the primary tumour. In addition, around half of the patients received the same first-line adjuvant FOLFOX chemotherapy after surgery. The homogenous patient characteristics and treatments, as well as the low rate of patient loss during follow-up, greatly reduced the confounding effects of the heterogeneous therapeutic modalities in most cancer clinical outcome studies.

Our study also has limitations. First, our sample size may not be large enough to detect minimal associations and interactions. Also, given the exploratory nature of our study as well as the small number of analysed SNPs, we did not conduct multiple comparison corrections for the main effects and stratified analyses. However, the homogeneous patient characteristics and treatments in our population minimise the confounding effects and enhance our statistical power. In addition, our power will be even increased with the further rapid growth of our patient population. Second, there is a possibility of chance findings in our study because of the short follow-up time (median follow-up time, 14.2 months) and the relatively small percentage of recurrence and/or death

event. Although the continuing growth of population with a low rate of patient loss during follow-up will further enable us to obtain adequate statistical power for in-depth analyses, larger retrospective or prospective studies in independent populations are warranted to further confirm our findings.

Overall, we showed that genetic variants that were associated with CRC risk might also influence CRC prognosis. Further validations of these findings are needed using independent populations, high-density mapping and functional characterizations.

Conflict of interest statement

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2011.02.004.

REFERENCES

1. Ferlay J, Parkin DM, Steliarova-Foucher E. Estimates of cancer incidence and mortality in Europe in 2008. *Eur J Cancer* 2010;**46**(4):765–81.
2. Karim-Kos HE, de Vries E, Soerjomataram I, et al. Recent trends of cancer in Europe: a combined approach of incidence, survival and mortality for 17 cancer sites since the 1990s. *Eur J Cancer* 2008;**44**(10):1345–89.
3. Yang L, Parkin DM, Ferlay J, Li L, Chen Y. Estimates of cancer incidence in China for 2000 and projections for 2005. *Cancer Epidemiol Biomarkers Prev* 2005;**14**(1):243–50.
4. Yang L, Parkin DM, Li L, Chen Y. Time trends in cancer mortality in China: 1987–1999. *Int J Cancer* 2003;**106**(5):771–83.
5. Compton CC, Fielding LP, Burgart LJ, et al. Prognostic factors in colorectal cancer. College of American Pathologists Consensus Statement 1999. *Arch Pathol Lab Med* 2000;**124**(7):979–94.
6. Chang GJ, Hu CY, Eng C, Skibber JM, Rodriguez-Bigas MA. Practical application of a calculator for conditional survival in colon cancer. *J Clin Oncol* 2009;**27**(35):5938–43.
7. Ulrich CM, Robien K, McLeod HL. Cancer pharmacogenetics: polymorphisms, pathways and beyond. *Nat Rev Cancer* 2003;**3**(12):912–20.
8. Naccarati A, Pardini B, Hemminki K, Vodicka P. Sporadic colorectal cancer and individual susceptibility: a review of the association studies investigating the role of DNA repair genetic polymorphisms. *Mutat Res* 2007;**635**(2–3):118–45.
9. Spitz MR, Wu X, Mills G. Integrative epidemiology: from risk assessment to outcome prediction. *J Clin Oncol* 2005;**23**(2):267–75.
10. Wang YC, Lee HS, Chen SK, Chang YY, Chen CY. Prognostic significance of p53 codon 72 polymorphism in lung carcinomas. *Eur J Cancer* 1999;**35**(2):226–30.
11. Han JY, Lee GK, Jang DH, Lee SY, Lee JS. Association of p53 codon 72 polymorphism and MDM2 SNP309 with clinical outcome of advanced nonsmall cell lung cancer. *Cancer* 2008;**113**(4):799–807.
12. Wu X, Lu C, Ye Y, et al. Germline genetic variations in drug action pathways predict clinical outcomes in advanced lung cancer treated with platinum-based chemotherapy. *Pharmacogenet Genomics* 2008;**18**(11):955–65.
13. Zafereo ME, Sturgis EM, Liu Z, et al. Nucleotide excision repair core gene polymorphisms and risk of second primary malignancy in patients with index squamous cell carcinoma of the head and neck. *Carcinogenesis* 2009;**30**(6): 997–1002.
14. Yaylim-Eraltan I, Arikan S, Yildiz Y, et al. The influence of cyclin D1 A870G polymorphism on colorectal cancer risk and prognosis in a Turkish population. *Anticancer Res* 2010;**30**(7):2875–80.
15. Zhang W, Gordon M, Press OA, et al. Cyclin D1 and epidermal growth factor polymorphisms associated with survival in patients with advanced colorectal cancer treated with Cetuximab. *Pharmacogenet Genomics* 2006;**16**(7):475–83.
16. Jiang J, Wang J, Suzuki S, et al. Elevated risk of colorectal cancer associated with the AA genotype of the cyclin D1 A870G polymorphism in an Indian population. *J Cancer Res Clin Oncol* 2006;**132**(3):193–9.
17. Siena S, Sartore-Bianchi A, Di Nicolantonio F, Balfour J, Bardelli A. Biomarkers predicting clinical outcome of epidermal growth factor receptor-targeted therapy in metastatic colorectal cancer. *J Natl Cancer Inst* 2009;**101**(19):1308–24.
18. Normanno N, Tejpar S, Morgillo F, et al. Implications for KRAS status and EGFR-targeted therapies in metastatic CRC. *Nat Rev Clin Oncol* 2009;**6**(9):519–27.
19. Tomlinson IP, Webb E, Carvajal-Carmona L, et al. A genome-wide association study identifies colorectal cancer susceptibility loci on chromosomes 10p14 and 8q23.3. *Nat Genet* 2008;**40**(5):623–30.
20. Broderick P, Carvajal-Carmona L, Pittman AM, et al. A genome-wide association study shows that common alleles of SMAD7 influence colorectal cancer risk. *Nat Genet* 2007;**39**(11):1315–7.
21. Tenesa A, Farrington SM, Prendergast JG, et al. Genome-wide association scan identifies a colorectal cancer susceptibility locus on 11q23 and replicates risk loci at 8q24 and 18q21. *Nat Genet* 2008;**40**(5):631–7.
22. Jaeger E, Webb E, Howarth K, et al. Common genetic variants at the CRAC1 (HMPS) locus on chromosome 15q13.3 influence colorectal cancer risk. *Nat Genet* 2008;**40**(1):26–8.
23. Kent WJ, Sugnet CW, Furey TS, et al. The human genome browser at UCSC. *Genome Res* 2002;**12**(6):996–1006.
24. Siepel A, Bejerano G, Pedersen JS, et al. Evolutionarily conserved elements in vertebrate, insect, worm, and yeast genomes. *Genome Res* 2005;**15**(8):1034–50.
25. Pomerantz MM, Ahmadiyeh N, Jia L, et al. The 8q24 cancer risk variant rs6983267 shows long-range interaction with MYC in colorectal cancer. *Nat Genet* 2009;**41**(8):882–4.
26. Shima H, Hiyama T, Tanaka S, et al. Loss of heterozygosity on chromosome 10p14–p15 in colorectal carcinoma. *Pathobiology* 2005;**72**(4):220–4.
27. Sneddon JB, Zhen HH, Montgomery K, et al. Bone morphogenetic protein antagonist gremlin 1 is widely expressed by cancer-associated stromal cells and can promote tumor cell proliferation. *Proc Natl Acad Sci USA* 2006;**103**(40):14842–7.

28. Waha A, Koch A, Hartmann W, et al. SGNE1/7B2 is epigenetically altered and transcriptionally downregulated in human medulloblastomas. *Oncogene* 2007;**26**(38):5662–8.
29. Wang W, Spitz MR, Yang H, et al. Genetic variants in cell cycle control pathway confer susceptibility to lung cancer. *Clin Cancer Res* 2007;**13**(19):5974–81.
30. Runnebaum IB, Wang-Gohrke S, Vesprini D, et al. Progesterone receptor variant increases ovarian cancer risk in BRCA1 and BRCA2 mutation carriers who were never exposed to oral contraceptives. *Pharmacogenetics* 2001;**11**(7):635–8.